

Project 1019572

Mechanisms for the Reduction of Actinides and Tc(VII) in *Geobacter sulfurreducens*

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RESULTS TO DATE: Recent results towards hypotheses 1-4:

Hypothesis 1 The mechanism of the reduction of U(VI) and Cr(VI) has now been studied in detail. Cr(VI) is reduced by one-electron transfer reactions to Cr(III), via a cell-bound Cr(V) intermediate identified by EPR spectroscopy. Studies with a cytochrome c7 mutant demonstrate that the electron transfer chain includes this protein which may be the terminal reductase for Cr(VI). Potential mechanisms of inhibition of Cr(III) precipitation, involving complex formation with organic acids commonly used as electron donors for metal reduction in the subsurface have also been identified. We have also initiated a collaboration with computational chemists led by Prof Ian Hillier in Manchester, to model metal binding to cytochrome c7, and subsequent electron transfer from the enzyme to the metal quantum mechanically. We have spent considerable time developing techniques to monitor the oxidation state of key actinides in contact with cells and enzymes of *Geobacter sulfurreducens*, including preliminary studies using X-ray absorption spectroscopy at the Daresbury synchrotron facility. We have now shown that U(VI) is reduced via a similar cytochrome c7 mechanism that is involved in Cr(VI) reduction, and we are currently conducting EPR and EXAFS studies to identify U(V) intermediates potentially formed during U(VI) reduction via single electron transfer. In sharp contrast to our original hypothesis, Np(V) is not reduced by *G. sulfurreducens*, showing that there is an unexpected degree of specificity for actinide species in this organism. This surprising result may also give us considerable insight into the mechanism of reduction of other actinides of interest to the DOE, and we are currently preparing for experiments using Pu(VI), Pu(V) and Pu(IV). The overall aim of our experiments on U(VI), Np(V), Pu(VI), Pu(V) and Pu(IV) is to develop a unified model for actinide reduction by *Geobacter* species. We have now made very significant progress in this area.

Hypothesis 2 Cytochrome c7 and a suite of site directed mutants have been prepared by our collaborator Marianne Schiffer (ANL). In order to determine if the mutated proteins are able to reduce U(VI) and other metals, we have overexpressed the protein in anaerobic cultures of *E. coli*. Despite successful targeting to the periplasm, the wild type cytochrome c7 was unable to form part of an active electron transport chain in *E. coli*, and this approach to exploring the functionality of mutant derivatives of cytochrome c7 will not be useful in our studies. We would prefer now to work with cytochrome c7 and mutated derivatives in vitro, and assay the ability of the proteins to transfer electrons to target metals using low concentrations of hydrogenase as the electron donor. This technique has proved useful in studying electron transfer to metals (e.g. Cr(VI)) in other anaerobic bacteria.

Hypotheses 3 In the first 5 months of this project we showed, using biochemical and genetic approaches, that the enzyme responsible for Tc(VII) reduction in *G. sulfurreducens* is a NiFe hydrogenase localized in the periplasm. We are now preparing to purify this protein to determine if it is able to reduce other metals including U(VI) directly, as suggested from a range of physiological experiments conducted in our laboratory e.g. the observation of hydrogen-dependent U(VI) reduction in the mutant lacking cytochrome c7. We have also recently conducted studies using an analogous highly purified hydrogenase from *Desulfovibrio vulgaris*, confirming that this second cytochrome-independent mechanism for U(VI) reduction is indeed possible in close relatives to *Geobacter* species.

Hypothesis 4 A suite of mutants has been constructed by our collaborator Derek Lovley at UMASS Amherst. Given the impressive progress in this area by the Lovley laboratory, and recommendations from reviewers of our grant application, we have decided to focus on a detailed characterisation of currently available mutants with respect to their ability to reduce a range of metals and actinides

DELIVERABLES: Publications from this grant include Lloyd, J.R. and Renshaw, J.C. (2004) Microbial transformations of radionuclides: fundamental mechanisms and biogeochemical implications. *Metals Ions in Biological Systems* Volume 42 (in Press) Lloyd, J.R., Anderson, R.T., Macaskie, L.E. (2004) Bioremediation of metals and radionuclides. In *Bioremediation*? R. Atlas and J. Philp (Eds), Washington; ASM Press (In Press) J.Renshaw, J. C., Lloyd, J. R., Livens, F. R., May, I., Coppi, M. V., Lovley, D. R. and Glasauer (2004) Reductive precipitation of the nuclear fuel cycle contaminant Tc(VII) by *Geobacter sulfurreducens*. In: Verstraete, (Ed). *European Symposium on Environmental Biotechnology, ESEB 2004*, London: Taylor and Francis Group. p. 251-254. Lloyd, J.R., Lovley, D.R., Macaskie, L.E. (2004) Biotechnological application of metal-reducing bacteria. *Advances in Applied Microbiology* 53 85-128 Lloyd, J.R. (2003) Microbial reduction of metals and radionuclides. *FEMS Microbiology Reviews* 27 411-425 Lloyd, J.R., Leang, C., Hodges Myerson, A.L., Coppi, M., Cuifo, S., Methe, B., Sandler, S.J. and Lovley, D.R. (2003) Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in *Geobacter sulfurreducens*. *Biochemical Journal* 369 153-161

Several additional manuscripts are in preparation describing our work on the biochemistry of Tc(VII) reduction in *Geobacter sulfurreducens*, and the mechanism of actinide reduction by this organism.

Posters: Glasauer, S.M., J. R. Lloyd, J. C. Renshaw, A. U. Gehring and T. J. Beveridge (2003). Immobilization of metals at the cell wall of subsurface bacteria during anaerobic respiration. *Goldschmidt meeting*, Japan Burke, I., Boothman, C., Lloyd, J. R., Mortimer, R.J.G., Morris K. (2003). Biogeochemistry of Technetium. *6th International Symposium on Environmental Geochemistry*, Edinburgh Several additional poster presentations will be made at *Goldschmidt 2004* (Copenhagen) and the *ASM 2004 Annual meeting* (New Orleans).

Invited presentations: *European Symposium on Environmental Biotechnology, Ostend* (2004) *Institut de Biologie Structurale et Microbiologie, CNRS, Marseille* (2003) *British Association Annual Meeting, Salford* (2003) *Advanced Science Research Center, JAERI, Tokai, Ibaraki, Japan* (2003) *Gordon Conference on Applied and Environmental Microbiology, Connecticut* (2003) *School of Biological Sciences, University of Dundee* (2003) *School of Biological Sciences, University of Liverpool, U.K.* (2003).

With upcoming talks at: *Symposium on Interactions of Heavy Elements with Microorganisms, Tokai* (2004) *European Meeting for Biotechnologies, France* (2004) *Biometals 2004 Symposium, Munich* (2004)